We must bear in mind that in all these cases the individual, or his friends, have been aware that something was wrong for years, and, knowing its rate of progress after the disease manifests its presence, we may confidently reason that it had been existent a very long time before that, so to impair the organ as to produce troublesome symptoms. A proportionate time and effort must be given to its relief. We patiently and persistently treat a chronic conjunctivitis and its sequelæ, and are encouraged to continue by our observation of the structural changes resulting from our efforts, and we must be guided and encouraged in the same way while attempting to improve this condition. We cannot rely entirely on restored function as evidence, for these people are more miserable than the blind, when they become profoundly deaf, without the element of maddening tinnitus which is so often present, and which so wears on the physical strength and the mental equilibrium, ceasing neither day nor night, that it must be an important factor in the causation of insanity and suicide. From a humanitarian view the result striven for is great, and any one who has even temporarily suffered in this way can appreciate what it must mean to be without hope of relief—*incurable.*

BACTERIOLOGY.

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Of the methods of investigating bacteria, there are three that are most important, viz.: (I.) The study of their characters by the aid of the microscope; (II.) their investigation by means of cultivation; and (III.) the study of their relation to the living body. The chemistry of their products is also very important, but the methods of chemical analysis can be found in works on chemistry and need not, therefore, be detailed here.

I. Microscopical Investigation.

It is unnecessary to go into details as to the construction and the theory of the microscope, but I may mention the chief practical points in using it. The objects being minute and delicate, accurate illumination and fairly high angled lenses are necessary.

The lenses required are a low power ($\frac{1}{2}$ inch) for adjustment, an intermediate power ($\frac{1}{4}$ or $\frac{1}{6}$ inch) to get a general view of the relation of parts, and high powers for exact examination. Of the high powers, the oil immersion lenses are the best, for two reasons: in the first place they are high angled and give a good picture and more light, and in the second place an adjustment collar is not absolutely necessary, and thus much time and trouble are saved. For most purposes $\frac{1}{12}$ oil immersion is sufficient, but $\frac{1}{18}$ and $\frac{1}{25}$ may also be obtained. The latter lenses not having, as a rule, such a high angle of aperture as the $\frac{1}{12}$, do not really show more detail; they merely show the objects larger.

Dry and water immersion high power lenses must be provided with a correction collar which enables the front and back lenses of the combination to be approximated or separated according to the thickness of the cover-glass; and, as cover-glasses vary much in thickness, the lens must be corrected for every specimen. In correcting a lens a high eye-piece must be used so as to magnify the defects as much as possible. Some delicate object is fixed, such as a delicate line, a group of granules, etc., and the collar is moved in one direction till it is evident that the picture is becoming worse; it is then moved in the opposite direction till the same thing happens, and so backward and forward in gradually diminishing distances till at last the best point is determined. In the case of oil immersions, it is well, also, to have a correction collar, for the oil is never of exactly the same refractive index as the glass; but, unless for very fine work, it is not necessary to alter it when the best average position has once been ascertained.

The microscope stand should be very steady and provided with a coarse and fine adjustment. The fine adjustment should work smoothly, slowly, and equably, otherwise it will be a matter of great difficulty to bring the object into accurate focus when using a high power. This is a matter of great importance. In most microscopes where the movement is obtained merely by a screw and a spring, the fine adjustment is imperfect, the parts get dirty, and the spring does not act instantly and steadily; hence the lens shoots past the focal point and exact focussing with very high powers is almost out of the question.

In bacteriological work a mechanical stage is often of great advantage. This is particularly the case where the whole specimen must be thoroughly searched for bacteria, and also where one wishes to find and resume the study, at a future time, of any special bacterium or group of bacteria. For the latter purpose a finder is necessary, the usual plan being that the fixed part of the stage is graduated on two sides and the amount of horizontal and vertical movement is read off. By placing the specimen and the movable stage in the same position, the object will be brought into the field of the microscope. Another very good method is by using a Maltwood's finder. Rotary stage movement is not necessary.

The substage arrangements are of the greatest importance. A wide-angled condenser is essential and it should be, if possible, achromatic. The substage must be provided with a coarse adjustment by which the condenser may be raised or lowered, and for fine work a fine adjustment in addition is an advantage. There must also be screws for centring the condenser. Rotation of the substage in not necessary for bacteric work. Diaphragms of various sizes are required; one of the best forms of diaphragm being the iris diaphragm, by means of which the amount of light admitted can be varied with the greatest ease.

The proper arrangement of the illumination is one of the most important parts of microscopy, though often neglected by investigators. The first point is to centre the condenser. This is done by using the smallest diaphragm, and, after the specimen is brought into view with the low power object-glass, racking down the condenser till the ring of the diaphragm appears sharp. By means of the centring screws this ring is brought into the middle of the field. Having centred the condenser, use a larger diaphragm or none at all, and rack up the substage till the source of light is sharply in focus at the same time that the specimen is well seen. When working with lamplight it is easy to focus the source of light and to get the flame of the lamp quite sharp, but with daylight it is not so easy. In the latter case it is better to focus some object at a little distance off, such as the branch of a tree, a chimney, etc.—the focus will be almost the same for clouds or sky. The centring of the condenser may require alteration when fresh lenses are used, because the position of the axis of each lens when screwed on to the tube is not always exactly the same. Hence if, when working with lamplight the flame is seen to have moved to one side of the field when a new lens has been put on, do not alter the position of the lamp but bring the flame into the centre of the field by means of the centring screws. When using the higher objectives the condenser can be still more accurately focussed by means of the fine adjustment. The centring of the condenser requires to be done only at the beginning of the day and needs attention only when new lenses are used; the focussing of the condenser must be done for every specimen unless, indeed, slides of exactly the same thickness are used throughout. As the slides are thicker or thinner, so must the condenser be approximated or removed in order to bring the source of light to a focus on the specimen. It is thus evident that good microscopes must have a coarse adjustment for the condenser and also centring arrangements. Those which have the condenser fixed or only sliding, and no centring arrangements, are bad. Oblique illumination is not required in this kind of work.

As to the source of light, we may use daylight or artificial light. Of artificial light the best is a paraffin lamp. Each of these has its advantages. My own opinion is that for high powers lamplight is best; the source of light is fixed and the light can be accurately concentrated on the point under examination, and in this way one can get more light and can apply it more accurately than with daylight, especially if one employs the edge of the flame, which gives about four times more light than the flat flame. On the other hand, for low power work daylight is undoubtedly the best. It fills the field equally, not being more intense at one part than another, while with lamplight, if the edge of the flame is used, only a narrow streak of light is seen in the centre of the field and a general survey cannot be easily obtained, while at the same time the illumination at the one point is too intense. It must be remembered that in using a condenser a flat mirror should be employed.

Very convenient lamps for microscopical work are made by Messrs. Swift & Beck. They consist essentially of large flat reservoirs for the oil. The chimney is a metallic one with a space on one side into which an ordinary microscopic glass slide can be slipped. The chimney can be turned round so that the glass slip may be brought opposite either the edge or the flat flame. The advantages of these lamps are that having a large reservoir they burn for a long time; that the reservoir being flat the lamp can be lowered much more than usual; that the light passes through a flat instead of a curved piece of glass; that the rest of the chimney being darkened there are no reflections; that if the glass breaks a fresh glass is always at hand and that the edge or flat flame can be used at will. The only difficulty about these lamps is that the glass is very apt to break. This may be avoided by selecting a thin glass and boiling it for some hours in water or oil before using it or by turning up the flame at first only very slowly. In using the lamp the light can be reflected into the microscope by means of a flat mirror, or the microscope may be tilted so that the light passes directly from the flame into the tube of the microscope, and this is the more satisfactory method when it can be employed. With lamplight it is well to interpose a piece of blue or neutral tint glass to remove the yellow color and make the light pleasanter to the eye. For this purpose colored glass of various intensities should be at hand.

I may sum up these remarks in the following rules:

1. Use a low power objective and a low eye-piece and place the specimen on the stage of the microscope.

2. Remove the diaphragm and rack up the condenser till it almost touches the slide.

3. Bring the specimen accurately into focus and leave it so.

4. Put in the smallest diaphragm and rack down the condenser till the hole of the diaphragm is sharply seen.

5. Centre the condenser by bringing this hole into the middle of the field. (4 and 5 need not be repeated more than once.)

6. Substitute a larger diaphragm and rack up the condenser till the source of light is accurately defined—the edge of the flame, branch of tree, etc. With lamplight it is best to dispense with the mirror and to place the lamp about eight inches from the condenser.

7. See again to the focussing of the specimen and be sure that it and the source of light are both at the same time accurately in focus.

8. Substitute the high power objective, again focus the specimen, and, if necessary, focus the light still more exactly. If the light (edge of the flame) is not quite in the centre of the field, bring it in by means of the centring screws, but do not move the lamp.

9. If the objective is a high dry power or a water immersion, it must now be corrected for the thickness of the cover glass. To do this, substitute a high eye-piece for the low one, and move the screw collar alternately in one or other direction till the sharpest picture is obtained.

The centring of the condenser (4 and 5) need only be done when one sits down to use the microscope, but the focussing of the condenser and the correction of the lens must be repeated with every new specimen. At first sight it may seem irksome to do this, but very soon one learns to do it quickly, and the gain in clearness, definition, and satisfactory examination of the specimen amply repays all the trouble.

In the investigation of bacteria the microscope is used for the examination of unstained and stained specimens. Of these the most valuable are the stained specimens, but as this involves the death of the organisms it is often necessary to study them without staining in order to see their normal form and structure, and to ascertain their characteristics, especially as regards the power of movement, mode of growth, grouping, etc.

A. Unstained Specimens.—In examining unstained specimens it is necessary to cut off a large amount of light by means of diaphragms, but the other arrangements of the microscope in respect of focussing and centring the condenser must be carried out in the manner just described. The bacteria to be examined without staining may be present in sections, or growing in fluid or in solid cultivating media.

It is but rarely nowadays that one examines bacteria in sections without staining them, but this was the method chiefly employed before the affinity of these organisms for the aniline dyes was discovered. In the unstained condition it is often difficult to distinguish the microorganisms from the tissue elements, and in order to do this with some degree of certainty the section is washed in acetic acid and then mounted in glycerine. Bacteria resist this treatment, while fatty and other materials are acted upon, and hence if groups of well-defined granules, all of the same shape and size, or of rod-shaped bodies are seen, they are in all probability microörganisms. Baumgarten succeeded in demonstrating the tubercle bacillus about the same time that Koch published his first paper on the subject, by subjecting sections of tuberculous tissue to the action of weak caustic potash; the tissue elements became invisible or less visible than usual, while the bacilli stood out markedly, having resisted the action of the alkali.

In examining pure cultivations of bacteria in fluids or solids, care must be taken not to let foreign bacteria into the cultivations from which the specimens are taken. The fluids may be obtained by using a thin platinum wire fixed in a glass rod and twisted into a loop at the end. The whole of the wire as well as the lower part of the glass rod must be carefully heated in the glass flame; when cool it is dipped into the flask, and a little of the fluid is picked up in the loop of the wire. Portions of fluid may also be removed by means of a sterilized pipette, the vessel containing the cultivation being held obliquely and the movements performed rapidly. Or, still better, a syringe may be employed, to the end of which a bent piece of glass rod is attached by India-rubber tubing; the glass rod is sterilized and introduced into the flask and a small quantity of the fluid drawn up by means of the syringe. If a small syringe with a ring at the end of the piston be employed, the whole manipulation can be done with one hand, the syringe being held in the hand and the piston withdrawn by means of the little finger passed into the ring.

The drop obtained by one or other of these methods may be examined by simply placing it on a slide and dropping a cover-glass on it. In this case care should be taken to have an air-bubble under the cover-glass, for it is often very difficult to focus a thin layer of fluid, while the margin of the air-bubble is easily defined and thus leads to the layer. This method is not sufficient if the examination is to be continued for some time, because evaporation occurs readily and also because there are very apt to be distracting currents, especially if an immersion lens be used. To avoid these inconveniences it is best to use cupped slides or glass cells. A small drop is placed on the middle of a large cover-glass, a little vaseline is put round the side of the cup or of the cell, the slide is turned upside down and dropped on the cover-glass so that the drop is in the centre of the cell, very slight pressure diffuses the vaseline and makes the cover-glass stick to the slide, and the slide is then quickly turned up so that the cover-glass is on the top with the drop hanging from its under surface. Where the observation of the drop is to be continued for some time, as where the mode of growth, spore formation, etc., is to be watched, the slide and cover-glass should be sterilized in the flame and the manipulations performed as quickly as possible. With regard to the cover-glass, care must be taken in passing it through the flame that it does not get too hot, otherwise it bends and does not lie flat on the top of the cell. If it is necessary to keep the cultivation, while under observation, at a temperature above that of the air, one or other form of hot stage must be employed instead of the cupped slide; but as the methods of using these are described in all books on microscopy, I need not go into that subject here.

Where the bacteria are growing on some solid material, a minute quantity is picked up with the sterilized needle and diffused in distilled water, salt solution (one-half to one per cent.), or cultivating fluid, and examined as above. It may also be necessary to examine bacteria *in situ* when growing on or in solid media, but this will be referred to afterward under "plate cultivations."

B. STAINED SPECIMENS.—The amount of information derived from the examination of unstained specimens is, however, comparatively small, and, therefore, in most cases recourse is had to the various methods of staining. It is only quite recently that satisfactorily stained specimens of bacteria have been obtained. The older attempts at staining were made with carmine and hæmatoxylin, but only a few species take on these stains, and in no case can the result be called good. With Weigert's discovery of the affinity of bacteria for certain of the aniline dyes, the state of matters has, however, been completely altered, and the work of Weigert, Koch, Ehrlich, and others, has led to great advances in this department of bacteriology. In resorting to stained specimens, the investigator may wish to study the bacteria alone, quite apart from their surroundings, or he may desire to ascertain their relations to the elements of the tissues in which they occur. In the former case what are termed "cover-glass preparations" are made; in the latter, sections of the tissue are employed. As the preparation and treatment of these two sets of specimens differ in several important particulars, it will be well to consider them separately.

1. *Cover-glass preparations.* These are employed when one examines bacteria in fluids, or in cultivations, etc., or when one wishes to gain an idea of the presence and probable numbers of these organisms in tissues without studying precisely their relations to the tissue elements. The method consists in spreading out the material in a thin layer on one side of a cover-glass, and fixing this layer to the glass. The material may be spread out by means of a needle, the point of a knife, etc., but the best way of obtaining an extremely thin and even layer is to take two cover-glasses, put a minute quantity of the fluid on the centre of one, drop the other glass on the top of it, press them together so as to diffuse the material over the glass, and squeeze out any superfluous fluid; then, keeping up the pressure, slide them apart and set them up on their edge to dry under protection from dust. They dry in a very short time, but if one wishes to hasten matters, they can be held in the fingers at a considerable distance above a flame; they must not, however, be actually heated while still moist, and by holding the glass in the hand instead of in forceps one takes care that they are not brought too near the flame.

In other cases the material may be too thick to spread out in the manner above mentioned; it must, therefore, be diffused in a drop of boiled distilled water. Where water is thus employed there is always the difficulty that after the cover-glasses are slid apart the water collects in drops as on a duck's back, and little heaps of microörganisms are obtained. It is therefore better when water is used not to press the drop between two cover-glasses, but to let it dry on one glass. It will be found that while at the centre there will probably be too many bacteria, at the circumference of the drop one will find satisfactory places. This is also the best way of preparing drop cultivations for staining The cover-glass from which the drop is hanging is turned over and the fluid allowed to dry. Where the material is very dense, as, for instance, a small tubercle, it is best to crush it well between two knives and then rub the crushed mass over the surface of a cover-glass, afterward removing the superfluous solid material. Lastly, we have what the Germans call "Klatsch" preparations, which are made in the following manner: As will afterward be pointed out, it is often important to study the mode in which various organisms spread over the surface of solid materials. When growth has occurred to some extent, a cover-glass is dropped over the spreading part, pressed lightly, and then lifted. The cover-glass picks up the bacteria, and when the specimen is dried and stained, one gets an exact impression of the mode of surface growth of the organism in question. These "impress preparations" are often very beautiful and valuable.

Cover-glasses prepared in any of these ways can be kept in a dry place for a long time without undergoing any change or losing their property of staining, and it is often necessary to keep a number of specimens of the same material either for future reference or for trying new stains or testing the activity of staining fluids which may have yielded negative results with other specimens. This is especially the case with tubercle. The stain ordinarily used for staining tubercle bacilli is very apt to go bad in a few days, and, therefore, I always keep a number of prepared cover-glasses of sputum or other material rich in tubercle bacilli at hand, so that if a negative result is obtained I can stain one of these glasses and see whether the result is due to some fault in the stain or to the absence of bacilli.

A layer having thus been obtained, it must be fixed before proceeding with the staining. If the layer contains albumen, the albumen is not coagulated by simple drying and, therefore, when placed to stain in a watery solution the albumen dissolves and the layer becomes detached. And even if any portion of the layer remains, that is not of much use, as precipitation of the stain occurs in connection with the albumen. It is, therefore, necessary to coagulate the albumen before proceeding with the staining and at the same time to retain for bacteria and nuclei their power of taking up the dye. This was done by Koch at first by placing the cover-glasses for some days in absolute alcohol. But this is a tedious method and one of the great advantages of these cover-glass preparations is that they can be prepared and stained in a very short time. Ehrlich found that if cover-glass specimens of blood were kept for an hour at 100° C. to 115° C. the desired coagulation was obtained without interfering with the staining of the nuclei, etc. Koch has applied this method to bacteric work, and at first recommended placing cover-glasses for an hour in an iron chamber kept at about 110° C. Ehrlich's method is simpler. He takes a long strip of metal, supports it on a tripod, and places a Bunsen's burner under one end. After about a quarter of an hour a wet rod is drawn over the metal from the cool end toward the flame. At a certain point it will be seen that the water boils. That line is, therefore, at 100° C. and its exact position is carefully ascertained. Cover-glasses with the coated surface upward are laid on that line or a little nearer the flame and kept there for an hour. As the best temperature varies for different materials, it is easily ascertained by using three or four cover-glasses and laying them at slightly different distances from the flame. One of them will be good.

Where one wishes to obtain really beautiful specimens for preservation the above is the best method, but it has been found that sufficiently good specimens are obtained by seizing the cover-glass in a pair of forceps with the spread side upward and passing it fairly rapidly three times through a glass flame (about as rapidly as one cuts bread—Rindfleisch). If it is passed too often through the flame, or if it is heated too long on the metal plate, the bacteria lose their property of taking on the stain.

Having thus prepared the specimens they must next be stained. This is much easier to do in the case of cover-glass preparations than in that of sections, but different kinds of bacteria stain differently. One of the most satisfactory stains to use is a fairly strong watery solution of methyl-violet. I do not make it up on any precise formula, but add a stock saturated alcoholic solution of methyl-violet to distilled water till a deep color is obtained. I should guess that the proportions are about one part of the methyl-violet to ten parts of water. A few drops of this are filtered into a watch-glass and then the cover-glass being held between the finger and thumb with the covered side downward, it is dropped on the stain and allowed to float there for five or ten minutes. It is then removed and washed repeatedly in dilute acetic acid (one-half to one per cent.) till no more stain comes out. It is afterward rinsed in distilled water and examined in the water. If a permanent preparation is required, it is then dried in the air and when quite dry is mounted in Canada balsam. If it is dried at a high temperature, the stain disappears and this is the case with all stained specimens; they should not be heated after staining. The specimens should always be examined in water in the first instance, because there the bacteria swell out to their natural size (more especially when acetic acid is used), whereas they dwindle and shrink somewhat when dried and mounted in Canada balsam. The use of acetic acid in the manner described above is much more satisfactory than that of alcohol for removing the superfluous stain and differentiating the nuclei and bacteria, and if the specimen is somewhat overstained to start with there is no danger of leaving it too long in the dilute acetic acid.

The Canada balsam is best employed in the form of a solution in xylol. The only other solvent that can be used is turpentine, as chloroform and benzol extract the aniline colors. Undissolved balsam cannot be used, for the heat required to dissolve it is apt to remove the stain from the bacteria.

Gentian-violet may also be employed in the same manner, or in a one per cent. watery solution, but the color is hardly, I think, so pleasant as that of methyl-violet, and for cover-glass preparations it does not possess any advantage. One of the quickest stains is a watery solution of fuchsin. This should be almost saturated and well filtered. If it is quite saturated precipitation is apt to occur on the specimen as evaporation takes place. The cover-glass is laid down with the spread side uppermost and a drop of the stain is placed on it. After about a minute the stain can be washed off with distilled water or weak acetic acid and the specimen examined.

Methylene-blue is almost more universally useful than methyl-violet, but the stain is not so intense. It may be used in the form of a strong watery solution, or it may be made in the same way as methyl-violet by the addition of a saturated alcoholic solution to water, but in larger proportions than in the case of methyl-violet. The watery solution stains a light blue or almost a green, but a beautiful deep blue is obtained if a little methylic alcohol be added to the solution, or if the saturated alcoholic solution be made with methylated spirit instead of with absolute alcohol. After staining in methylene-blue acetic acid should not be employed; simple washing with distilled water is sufficient.

The staining power of these solutions may be increased in two ways: either by warming the solution, or by the addition of various bodies, chiefly alkalies, and the alkaline solutions also act more quickly and powerfully when warmed. These facts were first made out in connection with Koch's discovery of the tubercle bacillus, and have since been extended to other bacteria.

Koch's first method of staining the tubercle bacillus was by the use of an alkaline solution of methylene-blue. He first made a watery solution of caustic potash of the strength of 1 to 10,000. To every 200 c. cm of this solution he added 1 c. cm of a saturated alcoholic solution of methylene-blue. The cover-glasses, which are prepared from tenacious pieces of yellow sputum or from crushed tubercles, are floated on this solution for twenty-four hours or longer, and are then placed in a saturated watery solution of vesuvin or Bismarck-brown for twenty to thirty minutes. They are then washed in water and examined at once or dried and mounted in Canada balsam. As the result of this treatment all bacteria, nuclei, etc., become in the first instance intensely blue, but the effect of the vesuvin is to substitute itself for the blue in the cell nuclei and in all the bacteria except the tubercle bacilli. The effect is that we have blue-stained tubercle bacilli on a brown ground. This method is, however, somewhat difficult to carry out, and it is somewhat difficult to see the tubercle bacilli, hence the plan was very soon improved.

The first improvement and that which still holds the field, was the use by Ehrlich of aniline oil instead of alkalies. Ehrlich's original plan was to make a watery solution of aniline oil by shaking up the oil with water (about three per cent. of the aniline is taken up) and filtering the turbid mixture through a moist filter. To this was added a saturated alcoholic solution of fuchsin, methyl-violet, or gentian-violet, till precipitation began to occur. Weigert has worked out the best proportions in which these two should be mixed, and gives the following formula:

Saturated watery solution of aniline	100 c. cm.
Saturated alcoholic solution of fuchsin, methyl-violet, etc.	11 "

The cover-glass is floated on this solution for about twenty-four hours at the ordinary room temperature, but three or four hours are quite sufficient at the body temperature, and if the staining fluid is heated almost to boiling a few minutes are enough. The most satisfactory result is obtained when the material is kept at the body temperature for two or three hours. When the cover-glass is removed from the staining fluid the whole layer is intensely stained and the individual structures unrecognizable. It is now immersed in dilute nitric acid (1 part of strong nitric acid to 3 or 4 parts of water) for a few seconds till the layer becomes bleached. The result of this is that while the stain remains fixed in the tubercle bacilli it is, so to speak, loosened in the other elements and easily removed by means of alcohol, or displaced by a contrast stain. If the cover-glass is washed in water after being taken out of the nitric acid the color returns (red or violet, according to the stain used) and is then less easily displaced. Hence some advise that the cover-glass should be placed at once in alcohol not quite absolute, after being removed from the nitric acid, but my own impression is, that it is better to wash away the acid first in water, as acid alcohol is apt to remove the stain from the bacilli unless they are very well stained. In any case, when the cover-glass is placed in alcohol it is left till no more stain comes out, and is then stained with a contrast stain, either a watery solution of methylene-blue made up as before described, if the primary stain was fuchsin, or a saturated watery solution of vesuvin if the primary stain was violet. When sufficiently stained they are washed in water and examined, or dried and mounted in balsam. The result is that the tubercle bacilli are red or violet as the case may be, and the nuclei of the cells and the other bacteria are blue or brown. Treated in this way, the only other bacteria, so far as is yet known, which retain the primary stain are the bacilli of leprosy. The spores of some large bacilli retain the primary stain, and elastic tissue and cheesy matter, especially the former, also retain it to some extent.

Stains made up according to the above formula become bad in a few days and must therefore be frequently renewed. This difficulty has been got over by avoiding the use of alcohol altogether, and the fluid now used in Koch's laboratory for Gram's method, tubercle bacilli, etc., is made up as follows: A saturated watery solution of aniline oil is made and filtered as above. This solution is then employed for preparing a saturated watery solution of fuchsin, methyl-violet, or gentian-violet, a large quantity of crystals of these substances being put into a bottle containing the aniline water. The fluid is filtered before use, and when exhausted fresh aniline water is added as long as any crystals remain at the bottom. This staining fluid remains good for a long time.

The mode of action of the aniline is not very clear. Ehrlich used it on the supposition that the difference in staining reactions between ordinary bacteria and tubercle bacilli depended on the tubercle bacilli possessing a sheath which can only be penetrated by coloring matters in the presence of alkalies, and which becomes impenetrable after the use of acids. But there are various facts which render it doubtful whether alkalinity is the essence of the matter. My own idea is that the aniline acts merely as a sort of fixing agent. I found[1] in my investigations on tuberculosis that the tubercle bacilli could be stained by the ordinary dyes without any aniline, but that only a few of them, probably at a certain stage of growth, could retain the stain in the presence of strong nitric acid. This is what the aniline enables them in some way or other to do. Other investigators, especially Baumgarten, have also found that these bacilli stain without the aniline. And even in the case of other bacteria the addition of aniline to the stain is an improvement, and indeed almost an essential in some instances.

Other substances have been employed to replace aniline, and with a considerable degree of success. The chief of these is a five per cent. solution of carbolic acid, first suggested by Dr. Franz Ziehl. Neelsen has modified Ziehl's solution and suggests the following:

Fuchsin	1 gramme.
Absolute alcohol	10 c. cm.
5 per cent, watery solution of carbolic acid . . .	100 c. cm.

This is used in the same manner as the Weigert-Ehrlich solution, or the stained cover-glasses may be washed in a watery solution of sulphuric acid (25 per cent.), instead of in nitric acid. The carbolic stain gives very brilliant results. Ziehl also succeeded with resorcin and pyrogallic acid, but the results are not so good. I have tested other substances, the best of these being phloroglucine, which acts almost as well as aniline. Toluidine is also good. Weigert recommends the following solution:

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[1] Practitioner, April, 1883.

Liq. ammon. fort.	5 c. cm.
Absolute alcohol	10 c. cm.
Distilled water	90 c. cm.
Gentian-violet	2 grammes.

This is used in the same manner as the others.

Gram's method of staining bacteria gives beautiful results in some instances, but it is not often necessary to use it in cover-glass preparations. Hence we may leave its detailed description till we come to sections, and the same may be done with regard to the other special methods, such as those for syphilis, double staining, etc.

Staining with vesuvin or Bismarck-brown was at one time much used in order to obtain specimens for photography, but since the introduction of the new plates by which blue and red stained specimens may also be photographed, this has fallen into disuse. One way of using the brown is to stain in a saturated watery solution of vesuvin, wash in water and examine, or dry and mount in Canada balsam. The method recommended by Koch was, however, to make a concentrated solution of vesuvin or Bismarck-brown in equal parts of glycerine and water, filtering before use. The specimens were afterward mounted in glycerine.

Quite recently it has been found that spores which, when completely formed, remain unstained when treated with the ordinary aniline dyes, can be stained by a special method. Koch and Gaffky noticed, when staining material containing tubercle bacilli with aniline methyl-violet, that not only the tubercle bacilli, but also the spores of a large bacillus retained the blue, while the other bacteria, and indeed the bacilli containing these blue stained spores, took on the brown stain. Hence there was here a differential staining of spore and adult bacillus. By using the same method, however, the spores of other bacteria could not be stained, but Neisser succeeded in getting double staining in other cases by using warm aniline fuchsin, and subsequent staining with methylene-blue; the spores remained red, the rest of the rod became blue. Buchner and Hueppe have worked out the matter, and Hueppe has obtained both isolated staining of spores and double staining of spore and rod. The isolated staining of the spore is managed in this way: if the cover-glass is passed through the flame oftener than three times, the staining property of the bacteria and protoplasm is diminished, and after passage through the flame six or ten times they do not stain at all. On the other hand, after the preparation has been passed through the flame from seven to ten times the spores acquire the property of staining with watery solutions of the basic aniline dyes, so that an isolated staining of the spores is the result. Hueppe also obtains double staining in the following manner: The cover-glass is passed through the flame three times, and then stained for ten to twenty minutes in a warm solution of aniline fuchsin (as used for tubercle). After removal from this solution it is washed in water, and then either stained with methylene-blue or placed for a few seconds in absolute alcohol, and then in the contrast stain. The spores come out red, the bacilli blue.

The flagella with which some bacteria are provided are said not to take on the aniline dyes, though I doubt if this statement is correct in all cases. On the other hand, they can be stained in a concentrated watery solution of hæmatoxylin.

Much more difficult than these cover-glass preparations is the manipulation of sections of the tissues. In this connection we have to consider the methods of hardening, of cutting, and of staining.

2. *Sections of the fresh tissues* may be made by means of freezing microtomes without any preliminary hardening, but the sections are not easily manipulated and in many cases the staining is most unsatisfactory. Hence it is best, whenever possible, to harden the tissues. If the fresh tissue is cut, the sections should be received in salt solution (one-half per cent.), and then placed by means of a spatula in absolute alcohol for a few minutes. From this they come into the staining fluid, and the best, for this purpose, is a saturated watery solution of vesuvin.

Of the hardening reagents the most useful is absolute alcohol, but Müller's fluid or a strong watery solution of bichloride of mercury may be employed. The tissues must be cut up into small pieces and placed in a large quantity of absolute alcohol, which should be frequently changed. Treated in this way the tissue is sufficiently hardened, and, indeed, in its best condition for staining in about three days. After several weeks' immersion in alcohol some bacteria do not stain so well as at first, and hence to keep the tissues permanently one should not use absolute alcohol, but a weaker alcohol: methylated spirit does very well for this purpose. In Müller's fluid (potassium bichromate, 2 grammes; sodium sulphate, 1 gramme; distilled water, 100 c. cm.) the tissues must be kept for from two to three weeks and are then ready for cutting, or after removal from Müller's fluid they may be placed in dilute alcohol and then transferred to and preserved in strong alcohol.

The tissues hardened in alcohol are best cut with a microtome, either a freezing microtome, or one for cutting under alcohol. If a freezing microtome is used, the alcohol must be first removed from the tissue by placing it in a large quantity of water. At first the tissue floats but after a short time it sinks, and in an hour or two it may be placed in a thick solution of gum. It remains in this for some hours to allow the gum to soak thoroughly into it. It is then frozen with either an ice or ether freezing machine. The best freezing microtome is, in my opinion, Williams's, and this can be easily arranged to act with ether. If ice is used, a few drops (six or eight drops per ounce) of methylated spirit should be added to the gum to prevent it freezing too hard, but with the ether machine this is not necessary as it is quite easy to regulate the degree of hardness of the gum. When cut the sections are placed in tepid water to dissolve out the gum, and are then removed to alcohol before being placed in the stain. In the case of Müller's fluid, the tissues may be placed at once in gum and then treated in the same way as the other.

At one time I used to prepare all the sections in this way, by first hardening the material in alcohol and then freezing, because very fine sections were thus obtained, but though this plan answers very well in the case of some bacteria, such as tubercle bacilli, I have come to the conclusion that on the whole a better result is obtained if the tissues are not placed in water after hardening, but are cut under alcohol. And after all, though the sections are a little thicker, this is no disadvantage, for one is less likely to miss the bacteria if they are few in number than when a very thin section is employed. For this purpose Thoma–Jung's microtome is the best. The bits of tissue are fixed on pieces of cork which can be grasped by the clamp of the microtome. The specimens are easily fixed on the cork by means of a mixture of gelatine and glycerine prepared in the following manner: Dissolve 5 grammes of gelatine in 10 c. cm. of distilled water, and then add 20 c. cm of glycerine. A minute portion of this is melted by heat and placed on a smooth piece of cork of suitable size and shape; on this the piece of tissue, from which the superfluous alcohol has been sucked off by means of blotting paper, is placed and the cork is then put into absolute alcohol. The glycerine jelly becomes quite firm after being left for a few hours in alcohol. Where the tissues are friable they must be embedded in paraffin or celloidin, best in celloidin. The tissue after having been hardened in alcohol is placed for some hours in a mixture of alcohol and ether. It is then removed to a solution of celloidin in equal parts of alcohol and ether and left for some hours. It may then be fixed on a cork by means of celloidin or laid in a paper mould filled with celloidin. In either case the specimen with its coating of celloidin is placed in an eighty-two per cent. alcohol to harden and is then cut as before. After the sections are cut they are put into alcohol.

The methods of staining differ for different bacteria, but are very similar to those described under cover-glass preparations. Where sections are being examined for unknown bacteria, the best solution to employ is probably Löffler's alkaline methylene-blue. The formula is:

100 c. cm. of a 1 to 10,000 solution of caustic potash.
30 c. cm. of a saturated alcoholic solution of methylene-blue.

The sections are left in this solution for from half an hour to an hour, and are then washed in distilled water or in dilute acetic acid (one-halt per cent.). If they are washed in acetic acid they must again be put in water to remove all the acid. They are then placed in alcohol to remove the water, then in oil of cedar, in which they may be examined or from which they may be mounted in Canada balsam dissolved in xylol. Oil of cloves should be avoided in all cases, as it is apt to dissolve out the stain from the bacteria.

The original method introduced by Weigert and Koch was to stain in a saturated watery solution of fuchsin, gentian-violet, or methyl-violet, or in a solution made as before described by adding a saturated alcoholic solution to water. From this the sections were transferred to water and then, in order to have only nuclei and bacteria stained, to dilute acetic acid; afterward to water again, alcohol, oil of cedar, etc. The acetic acid is, however, very apt to remove the stain from the bacteria, and hence it has been found best to give up acetic acid and use alcohol for differentiating the nuclei and bacteria. Thus, after being washed in water, they are at once placed in alcohol.

It is better when employing these stains to use the solutions in aniline water, because a deeper stain is thus obtained, and also because more bacteria take on the stain than when the plain watery solutions are used. The best stain is the saturated solution of gentian-violet, fuchsin, etc., in aniline water. This stains in fifteen to thirty minutes and the sections may or may not be washed in dilute acetic acid.

In sections stained in this way a double stain may often be obtained. Sections stained in aniline gentian-violet and washed in alcohol may be placed for a short time in a dilute alcoholic solution of eosin, and then again washed in alcohol and placed in oil of cedar. The result is that the bacteria and nuclei are blue and the remainder of the section rose-colored. In the same way violet stained specimens may be placed in various carmine solutions (chiefly picrocarmine), when the nuclei come out red and the bacteria violet, or specimens colored red may be stained in hæmatoxylin. In these cases, however, it is best to employ Koch's method of obtaining isolated staining of the bacteria before staining in the contrast stain. This is done by placing the sections, after being stained, in a solution made of equal parts of a saturated solution of potassium carbonate and distilled water. They remain there five minutes and are then transferred to alcohol.

One of the most beautiful methods of staining is Gram's method, but, unfortunately, only a few bacteria stain in this way. The sections are stained either in the Weigert–Ehrlich tubercle solution or in the saturated aniline solution of gentian-violet. Twenty to thirty minutes suffice. They are then at once placed in a solution of iodine (1 part of iodine, 2 parts of potassium iodide, and 200 parts of water) for five or fifteen minutes. Thence they are transferred directly to alcohol and left there till no more stain comes out. (It is frequently necessary to change the alcohol several times.) The result is that the section has a faint yellowish tinge while the bacteria appear of a blackish-blue color. These sections can then be double-stained by immersing them for a short time in a dilute solution of vesuvin, or, better, by placing them in a solution of picro-lithium carmine (1 part of lithium carmine and 3 parts of picric acid). In fact, the best way is to stain the sections first in picro-lithium carmine, then wash them in alcohol, and then stain with Gram's method; the nuclei and tissues retain the picrocarmine while the bacteria take on the gentian-violet. In this case care must be taken that the picrocarmine is fresh, for bacteria are very apt to grow in it, and, if that has occurred, when the specimen is afterward put in Gram's solution the bacteria adhering to it are stained, and mistakes are thus apt to occur. In staining with Gram's method the sections must not be put in water at all, and, indeed, it is important to remember in all these methods that it is best to put the sections straight from the alcohol in which they were first placed, after being cut, into the staining fluid and not to wash first in water.

Tubercle bacilli are stained in the same manner as described before under cover-glass preparations. The aniline fuchsin is the best solution to use, as the preparations are more permanent than those stained by gentian-violet. Various attempts have been made to get rid of the nitric acid, as it is apt to spoil the sections if they are very thin. In my paper on tubercle I suggested the following method: After staining the sections in Weigert-Ehrlich's fuchsin solution, wash in alcohol, and then place in the following:

Distilled water	100 c. cm.
Saturated alcoholic solution of methylene-blue . .	20 "
Formic acid	10 minims.

The sections are left for one or two hours in this solution and then washed in water, alcohol, etc. This method is not so certain as the older plan and, therefore, I only suggest its use for very delicate sections which are apt to be spoilt by the nitric acid.

Orth uses acidulated alcohol instead of nitric acid (one part of hydrochloric acid to 100 parts of 70 per cent. alcohol). Neelson employs dilute sulphuric acid as mentioned before. Other workers also employ acidulated alcohol. In fact, the acid is not absolutely necessary, for the contrast stain will ultimately replace the first stain in everything but the tubercle bacilli if it only acts long enough; indeed this was what occurred in Koch's original plan with methylene-blue and vesuvin. Gibbes has suggested a double stain in which the bacilli become red and the tissues blue, but, unfortunately, this does not give thoroughly reliable results.

Leprosy bacilli stand midway between ordinary bacteria and tubercle bacilli in that they can be stained by the ordinary stains, more especially by methylene-blue, while they also have the same reactions as tubercle bacilli in resisting the nitric acid after staining with Weigert-Ehrlich's solution. In fact, this is the best way to stain them.

Lustgarten has described bacilli in sections of gummata and other syphilitic products which stain in a characteristic manner. His method is the following: the sections are stained in the Weigert-Ehrlich gentian-violet solution in the same manner as for tubercle bacilli. They are then placed for a few minutes in alcohol and then for ten seconds in a one and one-half per cent. watery solution of potassium permanganate From this they are transferred to a watery solution of pure sulphurous acid. The specimen becomes bleached in a few seconds. If it is not sufficiently bleached it is washed with water and put back in fresh permanganate solution for two or three seconds and then again into sulphurous acid. This is repeated till the specimen is completely bleached; it is then washed with water, alcohol, etc. The bacilli come out violet, the rest of the tissue is colorless. They differ from tubercle and leprosy bacilli, which are also stained by this method, in that the syphilis bacilli are decolorized by the nitric acid method. De Giacomi suggests the use, first, of a dilute, and then of a concentrated solution of ferric chloride instead of the potassium permanganate and the sulphurous acid. In the healthy smegma, Alvarez and Favel have also found bacilli which show the same reactions and are of similar appearance, and their statements have been confirmed by others.

Several other bacteria are difficult to stain, but the peculiarities of each kind will be referred to later. Typhoid bacilli are best stained with the alkaline methylene-blue, and so are the bacilli of glanders, which are very difficult to stain. The spirilla of relapsing fever stain in cover-glass preparations of blood with methyl-violet, fuchsin, etc., but in sections the best solution is a watery solution of vesuvin or Löffler's methylene-blue solution.

For fungi in the tissues the methylene-blue solution is the best. For actinomyces Weigert recommends a solution of orseille dissolved in a mixture of 20 c. cm. of absolute alcohol, 5 c. cm. of acetic acid, and 40 c. cm. of distilled water, till the fluid is dark red. The sections are stained in this for an hour, then washed in a one per cent. watery solution of gentian-violet, and afterward in alcohol, oil of cedar, etc.

The rays of the fungus are stained bright red and the nuclei of the tissue violet.

In examining stained specimens with the microscope the focussing of the light, the centring of the condenser, and the correction of the lens must be carried out as carefully as in the other case, but instead of a small diaphragm a large one or no diaphragm at all must be employed. The reason is this: in the unstained specimen one wants to see as much of the structure picture as possible and, therefore, light must be shut off; but in stained specimens the stained objects would only be obscured by the structure picture, whereas they are quite as easily recognizable in a flood of light, while the structure picture disappears. The only objections to the entire absence of a diaphragm are, first, that if the light is very intense it is painful to the eye, and secondly, that it is only a very good lens that can stand the complete absence of the diaphragm, for if the lens is imperfect the image appears blurred.

Before examining specimens mounted in balsam with an oil lens it is well to surround the margin of the cover-glass with some material impermeable to oil, such as shellac, and allow this to harden before use. Hence, the investigation of the specimen, in the first instance, is made with the specimens in oil of cedar, and only when it is desired to preserve the specimen is it mounted in Canada balsam.

INTESTINAL CONCRETIONS RESEMBLING SAND, AND ORIGINATING IN CELLS FROM THE BANANA.

By N. L. Bates, M.D.,
MEDICAL INSPECTOR U. S. N.

Obscure and puzzling cases may eventually prove to be unexpectedly plain. What has been done by one is liable to be repeated by others; hence the history of my cases and of the efforts made to ascertain the nature of the unknown substance which caused them may save time and trouble to some one who might otherwise become involved in a search similar to my own.

Four years ago I washed from the fecal movement of a child a half ounce or more of siliceous, organic cells, closely resembling in external appearances fine black sand. Since that time these cells have been examined by many of the ablest chemists, microscopists, and physicians of the country, who were unable to give any information as to their origin, or even to decide whether they belonged to animal or vegetable life. Twice I read articles on the subject before societies; in one instance before a large audience representing almost every branch of scientific